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Fast Analysis of Total Polyphenol Content and Antioxidant Activity in Wines and Oenological Tannins Using a Flow Injection System with Tandem Diode Array and Electrochemical Detections

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Abstract

An analytical method for simultaneous determination of total polyphenol content (TPC) and antioxidant activity (AA) of wines (white and red wines) and oenological tannins, using a flow injection system with sequential diode array and electrochemical amperometry detectors (DAD-ECD), was proposed. The signal at 280 nm provided aggregate data for TPC. The anodic peak related to wine phenolic oxidation was scanned using pulsed integrated amperometry over the potential of 800 mV vs. Ag/AgCl, to obtain AA. Serial dilutions avoided the poisoning at the glassy carbon (GC) electrode and the linear response obtained with both detectors was compared with spectrophotometric assays commonly used in oenology laboratory. Intraday and interday analytical repetitions showed a good repeatability and reproducibility (relative standard deviation RSD < 6% for both detectors), and the satisfactory relationship between the proposed coupled flow injection/DAD-ECD and the classic UV methods (R^2_{TPC} = 0.9967; R^2_{DPPH} = 0.9621) confirmed the efficacy of flow injection analysis with a coupled detection system, for the reliable quality control of wine and wine-related products.

Keywords Total polyphenol content · Antioxidant activity · Electrochemistry · Flow injection · DPPH• · Method comparison

Introduction

Plant phenolic compounds are important secondary metabolites with antioxidant and antimicrobial activities, along with a great effect on the sensory properties of fruit and processed food and beverages (Haslam 1998). Wine is considered as one of the major source of phenolic compounds, including phenolic acids, flavan-3-ols derivatives, pigments, and proanthocyanidins (tannins) that are located in the solid part of the berry. The composition and properties of phenolic fraction in wine depends on their extractability, reactivity and solubility with time that are affected by many complex physico-chemical factors (Singleton and Esau 1969).

Spectrophotometric methods are commonly used in winemaking to determine the total polyphenol content (TPC) and their antioxidant activity (AA). However, the need for fast and combined analytical methods to monitor the evolution of the phenolic fraction of wine along the whole supply chain has stimulated the search of alternative approaches (Harbertson and Spayd 2006; Luque de Castro et al. 2005; Lorrain et al. 2013). Although spectrophotometry remains the classic tool to evaluate the polyphenol content, the measure of the antioxidant activity by electrochemical methods provides further insight into the redox ability of phenolic compounds that can be assimilated to the chemical electron transfer reaction and subsequent neutralization of reactive oxygen species occurring in wine (Kilmartin et al. 2001; Makhotkina and Kilmartin 2010). The selectivity of the electroanalytical methods can be tailored by varying the working potential values and its combination with the flow injection system represent a further potential improvement.

Although the chemical reducing mechanisms of polyphenols are likely to reflect the antiradical capacity of the wines (Arnous et al. 2002; Rivero-Pérez et al. 2007), and the electron transfer occurring in radical scavenging seems to exhibit

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similar mechanisms as the polyphenolic electrochemical oxidation (Arteaga et al. 2012; Gizdavic-Nikolaidis et al. 2004), the total antioxidant capacity of wines is derived from many variables; therefore, when testing new methods, a direct comparison is always needed.

The aim of this study was simultaneous determination of the total polyphenols and their radical scavenging activity by using a high-pressure, liquid injection system, which flow a small amount of sample in the diode array detector and subsequently in the amperometry cell, operating in pulsed (integrated) amperometry mode; we will refer to the system with the acronyms flow injection/DAD-ECD. The proposed flow injection method was applied to model solutions (gallic acid standard at different concentration levels) and real samples (wines (red and white) and commercial oenological tannins, both hydrolysable and condensed), in order to evaluate its efficiency in fast and reliable analysis of TPC and AA parameters in oenological samples, as an alternative to routinely used, time-expensive analytical methods.

Materials and Methods

Chemicals

Gallic acid monohydrate HPLC grade (≥ 98%) and the 2,2-diphenyl-1-picrylhydrazyl (DPPH•) were purchased from Sigma-Aldrich (Saint Louis, MO). Stock standard solutions of gallic acid were freshly prepared in water (Milli-Q ultrapure water, Millipore, Bedford, MA). Methanol was HPLC-gradient grade (< 99.8%) (VW, Radnor, PA). L-(+)-Tartaric acid had analytical grade (≥ 99.5%) and ethanol was with HPLC grade (≥ 99.9%), used for preparation of the mobile phase for HPLC analysis and the model wine solutions, respectively (Merck, Darmstadt, Germany).

Wines and Oenological Tannins

TPC and AA were determined in wines and commercial tannins. In total, 31 wines were analyzed, produced from various red and white grape varieties grown in Italy and Republic of Macedonia. Wine samples included 5 Cabernet Sauvignon, 12 Vranec, 4 Syrah, 2 Merlot, 1 Stanushina from different wine-growing zones in Macedonia, together with 1 Sauvignon Blanc, 1 Lambrusco Gasparossa, 2 Chardonnay/Trebbiano blends, 1 organic Chardonnay, and 2 Muller Thurgau from Italy. All wines were from 2012 vintage, with alcohol content ranging 10–14% for white wines and 12.5–16.4% for red wines. They were sampled following the end of the vinification process and stored in glass vials (40 mL), saturated with nitrogen and capped with plastic screw-cap prior to analysis; they were stored at room ambient (22 ± 1 °C), avoiding direct sun exposure. In addition, 12 oenological, commercial-grade tannins (HTS Enologia, Marsala, TP, Italy; Laffort, Bordeaux Cedex, France) supplied as lyophilized extracts from different botanical sources (oak, chestnut, gallnut, and grape) were dissolved in a model wine solution (i.e., ethanol 12% *v/v* and tartaric acid 2 g/l, pH 3.6) at concentration of 50 mg/L of powder for the analyses. Samples were filtered with 0.22 μm cellulose acetate membrane filter (WVR International, Radnor, PA, USA) prior to analysis.

Spectrophotometric Assays

The TPC of wines and grape tannins was determined at wavelength of 280 nm ($UV_{280\ nm}$ assay) using a 10-mm quartz cuvette (Ribéreau-Gayon 1970). The determination of polyphenols by mean of the optical density at 280 nm is a direct method to quantify polyphenols, and it provides a reliable value which is commonly used by the enological companies for fermentation/vinification monitoring.

Samples were diluted in model wine solution as follows: 100 dilutions for red wines, 10 dilutions for white wines, whereas tannin solution were analyzed at the working concentration of 50 mg/L. Results were expressed as millimolar gallic acid equivalent (GAE) against a gallic acid calibration curve over the range 0–0.18 mmol gallic acid/L (R^2 = 0.9972; intercept = − 0.0322; slope = 5.7378).

The radical scavenging activity was determined with 2,2-diphenyl-1-picrylhydrazyl (DPPH•) synthetic radical method (Brand-Williams et al. 1995), using 40-fold dilution for red wines and 5-fold dilution for white wines, in model wine solution; tannin solutions were analyzed at the working concentration of 50 mg/L. Samples were incubated for 1 h and the absorbance was measured at 517 nm with a 10-mm plastic cuvette against pure methanol. Results were expressed as mM GAE using a calibration curve in the range 0–0.15 mmol gallic acid/L (R^2 = 0.997; intercept = 0.8067; slope = −3.94). Samples were analyzed in duplicate using a Shimadzu UV mini 1240 spectrophotometer (Kyoto, Japan).

High-pressure Flow Injection Analysis

The flow injection system was adapted from a Dionex high-pressure liquid chromatography system equipped with GP50 gradient pump, a PDA-100 photodiode array detector (DAD) and an ED50 Electrochemical Detector (ECD) (Thermo Fisher Scientific Inc., Waltham, MA); detectors were connected in series between them and they were both plugged into the injection system through a peak line composed of two modules, bearing different inner sections; this allowed a stable counter pressure of 78 bar for the entire duration of analysis. The analysis was carried out as follows:

- Eluent: 50 mM tartaric acid aqueous solution
- Elution: isocratic flux for 3 min, with a flow rate of 1.0 ml/min

– Injection: 25 μl of diluted sample (red wine: 40-fold in distilled water; white wines: 5-fold in distilled water; tannins: stock solution).

The DAD was set up at 280 nm, the peak was integrated baseline (Fig. 1a), and the total polyphenol content was calibrated as GAE in the range 0–0.37 mM gallic acid/L (R^2 = 0.9994; intercept = 0.9607; slope = 201.38). The ECD was set at 800 mV potential to oxidize all the phenolic compounds that contribute to the antioxidant activity in wine (Mannino et al. 1998). The anodic current under the amperometry peak (Fig. 1b) was integrated to determine the total antioxidant activity of wine polyphenols using a calibration curve over the range 0–0.19 mM GAE (R^2 = 0.9984; intercept = 0.4633; slope = 189.41). The electrochemical analysis was performed in integrated amperometry mode, applying a waveform with cycles of 0.5-s duration, divided into three regions (Fig. 2): (i) E0-E1 concerned the absorption of the analyte at the electrode surface and initiation; (ii) E2-E3 involved the current integration period; (iii) E4-E6 involved the cleaning steps to remove passivation layer and activate the electrode surface. The cathodic cleaning of the glassy carbon (GC) electrode at − 2.0 V allowed to reduce the oxidation products that adsorbed at the electrode surface, and to release them in solution; it was followed by a rapid excursion to 0.6 V potential and then returned to the initial − 0.1 V value to reduce the remaining oxides formed at 0.6 V and rebalance the cell for a new integration cycle.

Samples were analyzed in duplicate and followed by injection of a tartaric acid solution to regenerate the flow system; the CG electrode was periodically cleaned and regenerated with a polishing kit consisting of a urethane fiber polishing pad and 0.3 μm alumina powder (Thermo Fisher).

Data Processing and Statistical Analysis

TPC and AA results obtained from both traditional spectroscopic and innovative proposed methods were stored and statistically processed by means of Analyse-it 4.20.1 for Excel (Analyse-it Software, Leeds, UK).

Results and Discussion

The proposed innovative analytical approach, providing an in-line simultaneous determination of total phenolics and antioxidant activity in wine and tannins was evaluated according to analytical performances and compared with standard spectrophotometric methods, i.e., $UV_{280\ nm}$ and DPPH•.

The flow injection method coupled with DAD-ECD detection was calibrated for both TPC and AA measurements using gallic acid as a standard (Table 1); validation showed satisfactory limit of quantification (LOQ = 0.012 mM (DAD); 0.005 mM (ECD)) that were suitable for the TPC and AA values expected in the oenological samples. The quantification

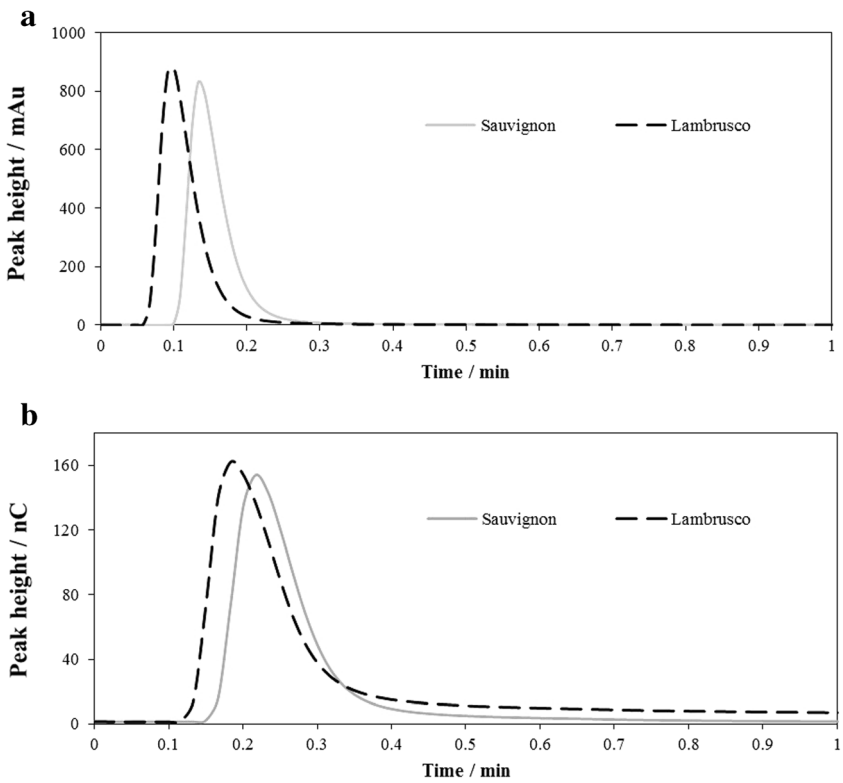

Fig. 1 Flow injection/DAD (**a**) and flow injection/ECD (**b**) signal of white wine (Sauvignon Blanc) and red wine (Lambrusco)

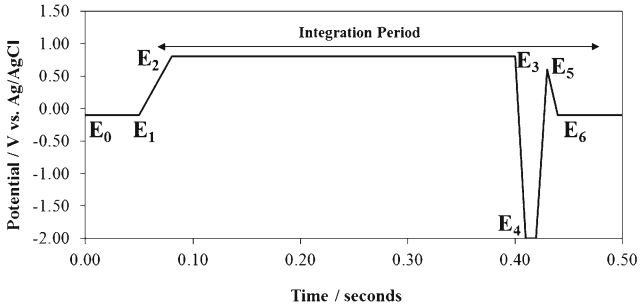

Fig. 2 Integrated amperometry waveform used to determine the antioxidant activity of phenolic compounds in wines and model wine solutions. Detector response is the charge from integration of the phenolic oxidation current between 0.20 and 0.50 s, expressed as nC

showed good agreement and a slight improvement with respect to previous alternative analytical methods developed to quantify polyphenols, which exhibited LOQ values ranging from 0.006 to 0.25 mM for the gallic acid standard (Arce et al. 1998; Gòmez-Alonso et al. 2007; Aid et al. 2015) and compared to the differential pulsed voltammetry method for the determination of antioxidant activity, which showed a LOQ of 0.006 mM in Catechin Equivalent, CE (Šeruga et al. 2011). Results also showed improved performances according to LOQ calculated for the Folin-Ciocalteu method to determine the TPC (LOQ = 0.013 mM GAE) and DPPH method to determine the AA (LOQ = 0.037 mM Trolox Equivalent, TE), both related to the microplates assays (Bobo-García et al. 2014). Direct determination of polyphenols by means of absorbance intensity at 280 nm was applied in this experiment, setting the wavelength value which is routinely used in both HPLC-DAD and spectroscopic quantifications of polyphenols, thus avoiding time-consuming colorimetric assays which were not applicable in this instantaneous flow stream analysis.

Table 1 Statistical parameters for the method calibration with gallic acid

	Flow injection-DAD	Flow injection-ECD
LOQ (mmol gallic acid/L)	0.012	0.005
Slope of the curve		
Average	190	188
SD	0.96	1.01
RSD (%)	0.51	0.54
Intercept of the curve		
Average	0.99	0.51
SD	0.16	0.06
RSD (%)	15.94	12.45
Coefficient of determination (R^2)	0.9994	0.9984

The phenolic composition of 43 samples, including 31 wines and 12 commercial tannins, analyzed with the flow injection/DAD-ECD and the spectrophotometric methods are presented in Table 2.

The TPC content exhibited a concentration range 1.48–21.3 mM GAE for wine samples, with average value of 9.3 mM GAE, and a concentration range 0.1–0.51 mM GAE for tannin solutions, with average value of 0.17 mM GAE when calculated using the $UV_{280\ nm}$ method; same samples showed a TPC content in the range 1.08–15.4 mM GAE for wine samples, with average value of 6.5 mM GAE, and a concentration range 0.07–0.34 mM GAE for tannin solutions, with average value of 0.12 mM GAE when calculated using the flow injection/DAD method.

The AA parameter exhibited activity values expressed in gallic acid equivalent activity in the range 0.35–6.52 mM GAE for wine samples, with average value of 3.19 mM GAE, and activity values ranging 0.07–0.16 mM GAE for tannin solutions, with average value of 0.09 mM GAE when calculated using the DPPH• method; same samples showed AA values in the range 0.42–6.04 mM GAE for wine samples, with average value of 3.27 mM GAE, and a concentration range 0.07–0.26 mM GAE for tannin solutions, with average value of 0.12 mM GAE when calculated using the flow injection/ECD method.

Concentration ranges were consistent with compositional data previously reported for the same wine varietals (Ivanova-Petropulos et al. 2015; Ivanova et al. 2011) and tannins (Magalhães et al. 2014).

Visual examination of results and linear regression allowed to detect a bias in the data as the regression lines were significantly different from slope (β) of 1 (Table 3). The Bland-Altman plot, used to compare the results of each and every two methods by plotting the absolute difference (method A: flow injection–method B: spectrophotometric) as a function of the measurements average [(method A + method B)/2] (Bland and Altman 1999), disclosed a significant trend between the methods except for comparison between AA (flow injection -ECD 800 mV) and AA (DPPH• assay) (*data not shown*). The finding imply the presence of a systematic proportional difference between the compared methods of measurement that was further confirmed by Deming's regression ($\beta \neq 1$). Under this condition, the paired *t* test is unsuitable for testing the relationship between the two methods. As the same standards were used in each system, the presence of systematic bias should not be attributable to the calibration procedure. Bias between spectrophotometric and electrochemical methods may be due to the nature of the measurement being more dependent on activity rather than concentration. Additional variability in the electrochemical detection can be introduced by the very short reaction time. Further possible sources of systematic bias can be nonadjustable instrumental bias and cell design.

Table 2 Sample dataset for comparison between total phenolic compounds (TPC) and antioxidant activity (AA), expressed in gallic acid equivalents (GAE) and calculated with the flow injection-DAD-ECD and spectrophotometric (UV_{280nm}, and DPPH•) methods, respectively

Sample	Flow injection DAD/ECD (mM GAE)		Spectrophotometric determinations (mM GAE)	
	TPC_DAD 280 nm	AA_ECD 800 mV	TPC_ UV 280 nm	AA _ DPPH assay
Wines				
Cab Sauvignon	7.25 ± 0.01	3.57 ± 0.07	10.4 ± 0.027	3.84 ± 0.07
Cab Sauvignon	8.93 ± 0.20	4.65 ± 0.14	12.2 ± 0.05	4.59 ± 0.00
Cab Sauvignon	9.07 ± 0.13	4.72 ± 0.04	12.7 ± 0.03	4.83 ± 0.01
Cab Sauvignon	3.59 ± 0.03	1.93 ± 0.04	5.92 ± 0.00	2.09 ± 0.03
Cab Sauvignon	7.31 ± 0.01	4.05 ± 0.03	10.5 ± 0.15	3.84 ± 0.06
Vranec	15.4 ± 0.03	6.04 ± 0.01	21.3 ± 0.32	6.52 ± 0.05
Vranec	11.1 ± 0.14	3.21 ± 0.49	15.5 ± 0.14	4.46 ± 0.40
Vranec	8.23 ± 0.08	3.83 ± 0.03	12.4 ± 1.4	4.15 ± 0.00
Vranec	6.37 ± 0.07	3.52 ± 0.04	9.81 ± 0.79	3.24 ± 0.01
Vranec	6.92 ± 0.08	4.03 ± 0.22	9.53 ± 0.27	3.53 ± 0.01
Vranec	10.6 ± 0.36	4.43 ± 0.13	15.6 ± 0.06	4.38 ± 0.17
Vranec	11.1 ± 0.06	4.92 ± 0.01	15.9 ± 0.06	5.31 ± 0.61
Vranec	7.89 ± 0.08	4.46 ± 0.12	11.2 ± 0.13	4.16 ± 0.04
Vranec	9.28 ± 0.45	4.55 ± 0.08	13.9 ± 0.07	4.75 ± 0.01
Vranec	9.67 ± 0.27	4.12 ± 0.19	13.9 ± 0.03	4.96 ± 0.10
Vranec	4.59 ± 0.01	2.99 ± 0.12	7.33 ± 0.15	2.58 ± 0.01
Vranec	4.31 ± 0.01	3.12 ± 0.05	6.42 ± 0.14	2.47 ± 0.14
Syrah	8.83 ± 0.08	4.33 ± 0.28	12.5 ± 0.21	4.01 ± 0.04
Syrah	6.91 ± 0.13	4.46 ± 0.21	9.37 ± 0.08	3.34 ± 0.01
Syrah	7.13 ± 0.07	3.53 ± 0.03	10.3 ± 0.02	3.64 ± 0.00
Syrah	6.98 ± 0.03	3.42 ± 0.01	10.0 ± 0.19	3.73 ± 0.01
Merlot	8.65 ± 0.11	4.97 ± 0.09	11.7 ± 0.03	4.35 ± 0.04
Merlot	8.03 ± 0.00	4.72 ± 0.07	11.2 ± 0.17	4.23 ± 0.02
Stanushina	6.11 ± 0.00	3.02 ± 0.08	8.97 ± 0.09	3.18 ± 0.04
Sauvignon Blanc	1.22 ± 0.00	0.42 ± 0.00	1.50 ± 0.21	0.36 ± 0.01
Lambrusco Gasparossa	1.28 ± 0.01	0.67 ± 0.04	1.62 ± 0.07	0.42 ± 0.01
Chardonnay/Trebbiano	1.43 ± 0.01	0.79 ± 0.05	1.67 ± 0.00	0.41 ± 0.08
Chardonnay/Trebbiano	1.10 ± 0.01	0.61 ± 0.08	1.51 ± 0.01	0.35 ± 0.00
Chardonnay Bio	1.25 ± 0.02	0.90 ± 0.07	1.64 ± 0.01	0.43 ± 0.10
Muller Thurgau	1.09 ± 0.00	0.82 ± 0.05	1.49 ± 0.00	0.42 ± 0.00
Muller Thurgau	1.08 ± 0.04	0.66 ± 0.05	1.48 ± 0.02	0.41 ± 0.05
Grape tannins				
Limousin oak tannin	0.10 ± 0.000	0.10 ± 0.021	0.14 ± 0.000	0.09 ± 0.002
American oak tannin	0.07 ± 0.000	0.07 ± 0.003	0.10 ± 0.001	0.07 ± 0.001
French oak tannin	0.08 ± 0.000	0.09 ± 0.001	0.12 ± 0.001	0.08 ± 0.000
Selected oaks tannin	0.07 ± 0.002	0.09 ± 0.003	0.11 ± 0.005	0.07 ± 0.001
American oak tannin	0.09 ± 0.001	0.10 ± 0.001	0.12 ± 0.001	0.07 ± 0.001
Chestnut tannin	0.14 ± 0.002	0.16 ± 0.009	0.19 ± 0.002	0.11 ± 0.002
American oak tannin	0.08 ± 0.001	0.09 ± 0.000	0.12 ± 0.001	0.07 ± 0.000
Selected oaks tannin	0.13 ± 0.002	0.11 ± 0.005	0.17 ± 0.002	0.10 ± 0.001
Gallnut tannin	0.34 ± 0.001	0.26 ± 0.001	0.51 ± 0.000	0.16 ± 0.000
Grape berry tannin	0.08 ± 0.001	0.10 ± 0.003	0.12 ± 0.002	0.10 ± 0.001
Oak heartwood tannin	0.14 ± 0.001	0.19 ± 0.003	0.19 ± 0.000	0.10 ± 0.001
Selected oaks tannin	0.11 ± 0.004	0.11 ± 0.002	0.14 ± 0.002	0.08 ± 0.001

Although the TPC values obtained with the two methods, i.e., $UV_{280\ nm}$ and flow injection/DAD, showed a good correlation ($R^2 = 0.9967$), the flow injection/DAD assay underestimated the results if we consider the $UV_{280\ nm}$ as a reference method (slope = 0.702) therefore a correction factor would be required to fit the results. This finding can be partly explained by the different approaches in extrapolating results, i.e., $Abs_{280\ nm}$ intensity (mAu optical density) in the UV assay and the peak area calculation (mAu density/unit of time) in the case of flow injection/DAD. Potential interferences, i.e., baseline modifications induced by chemical interferents in the $UV_{280\ nm}$ spectrophotometric method, have to be further investigated in future works.

The relationship between DPPH• and flow injection/ECD amperometry was satisfactory ($R^2 = 0.9621$, Fig. 3), with almost the same sensitivity (slope = 0.947); a slight divergence

Table 3 Linear correlations among TPC and AA parameters measured using spectrophotometric and flow injection methods and related statistical parameters

	$UV_{280\ nm}$	DPPH•	Flow injection/DAD	Flow injection/ECD
$UV_{280\ nm}$	1	$R^2 = 0.9792$ Slope = 2.93 MSE = 0.088 RMSE = 0.296 Standard error = 0.023	$R^2 = 0.9967$ Slope = 1.421 MSE = 0.063 RMSE = 0.251 Standard error = 0.009	$R^2 = 0.9234$ Slope = 2.949 MSE = 0.309 RMSE = 0.556 Standard error = 0.044
DPPH•	$R^2 = 0.9792$ Slope = 0.334 MSE = 0.088 RMSE = 0.296 Standard error = 0.023	1	$R^2 = 0.9767$ Slope = 0.475 MSE = 0.432 RMSE = 0.658 Standard error = 0.024	$R^2 = 0.9621$ Slope = 1.016 MSE = 0.152 RMSE = 0.390 Standard error = 0.031
Flow injection/DAD	$R^2 = 0.9967$ Slope = 0.701 MSE = 0.063 RMSE = 0.251	$R^2 = 0.9767$ Slope = 2.0562 MSE = 0.099 RMSE = 0.315 Standard error = 0.024	1	$R^2 = 0.9246$ Slope = 2.072 MSE = 0.308 RMSE = 0.555 Standard error = 0.044
Flow injection/ECD	$R^2 = 0.9234$ Slope = 0.3131 MSE = 2.905 RMSE = 1.704 Standard error = 0.044	$R^2 = 0.9621$ Slope = 0.9471 MSE = 0.162 RMSE = 0.403 Standard error = 0.031	$R^2 = 0.9246$ Slope = 0.446 MSE = 1.432 RMSE = 1.197 Standard error = 0.044	1

from linearity could be related to the different chemical mechanisms and selectivity acting during these assays, i.e., chemical in one case, electrochemical in the other. In particular, the stoichiometry of DPPH• is complicated as the redox reactions can be continued by the oxidation-degradation products the time response curve to reach the steady state is not linear with different ratios of antioxidant/DPPH; for example, tannic acid is able to reduce 50 mol of DPPH•, while caffeic acid reduces 2.6 mol only (Dicu et al. 2010). Moreover, the DPPH• assay is greatly affected by solvent impurities and change in pH (Danilewicz 2015), and the DPPH• radical scavenging is enhanced when working with alcoholic solutions due to a partial ionization of the phenols (Foti and Ruberto 2001; Litwinienko and Ingold 2003). Most of these limitations can be overcome by using electrochemical measurements, which allow a direct measurement of the current generated by the oxidation of phenolic compounds with no other reagents except the aqueous electrolyte.

The good correlation between the polyphenolic content and the calculated antioxidant activity values for each method ($R^2_{UV280\text{-}DPPH} = 0.9792$, and $R^2_{DAD\text{-}ECD} = 0.9246$) confirmed the origin of the antioxidant properties of wine that is greatly explained by the polyphenolic compounds, especially procyanidin oligomers (Muselík et al. 2007) and anthocyanins (Tenore et al. 2011). It is noteworthy that the reduction potential E_0 of the DPPH•/DPPH redox couple vs the standard hydrogen electrode (SHE) in pure methanol or methanol/water solvents (60:40, *v*/*v*) are + 0.47 V and + 0.45 V, respectively (Chen et al. 2011). As the Fe(III)/Fe(II) redox couple has been found to be reduced to + 0.385 V by cyclic voltammetry in model wine at

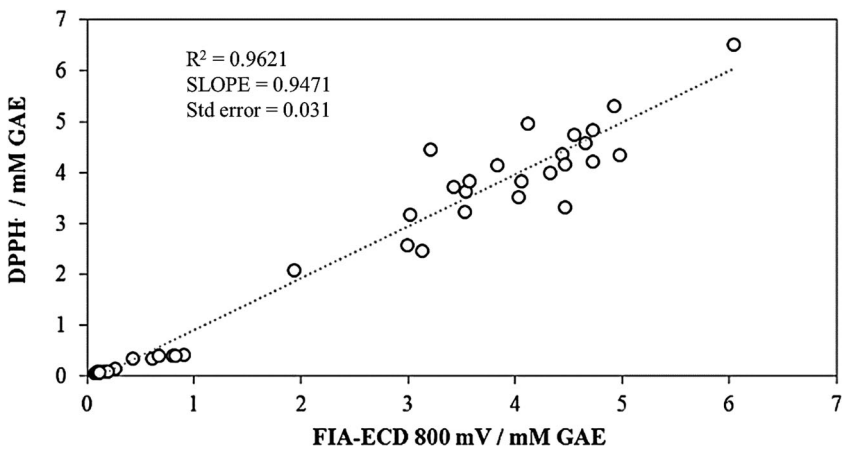

Fig. 3 Correlation between antioxidant activity (AA) assays: DPPH vs flow injection/ $ECD_{800\ mV}$

pH 3.30 (Danilewicz 2013); thus, thermodynamic of wine compounds should be similar in the two assays, which should lead to a good correlation between their results.

Conclusions

A new simple tool for the rapid and simultaneous determination of total polyphenols and antioxidant activity in wine and oenological tannins was proposed. The flow injection/DAD-ECD method was successfully applied for the direct and rapid measurement of the total phenolic content and the antioxidant capacity in wine. The performance of the flow injection method was compared with well-established spectrophotometric assays showed the following advantages: (i) the analysis was extremely fast (3 min required), when compared with time-consuming spectrophotometric assays; and (ii) it provided the determination of both TPC and AA parameters in the same analysis with high sensitivity, reliability, and repeatability. Both analytical approaches pointed out the dependence of wine protection against oxidation upon the content in polyphenolic compounds, even though it was observed that the DPPH• assay only accounted on the molecular features having faster kinetics of reaction against radical, while the electrochemical method allowed a full screening of the overall antioxidant activity. The total polyphenol content generally provided higher values when using the optical density at 280 nm, and this is probably due to the effect of interferents contained in wine which absorb at the same wavelength; the integration of the peak produced in the flow injection/DAD analysis seemed to provide a higher selectivity. In brief, the proposed approach combining both spectral and electrochemical detections generate a unique pattern for each samples, which is useful for classification and quality control.

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Compliance with Ethical Standards

Conflict of Interest Arianna Ricci declares that she has no conflict of interest. Nemanja Teslic declares that he has no conflict of interest. Violeta-Ivanova Petropolus declares that she has no conflict of interest. Giuseppina Paola Parpinello declares that she has no conflict of interest. Andrea Versari declares that she has no conflict of interest.

Ethical Approval This article does not contain any studies with human participants or animals performed by any of the authors.

Informed Consent Not applicable.

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